Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the investigation of **Clove Oil** in the developing chicken embryo 1/31/74

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of CLOVE OIL in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo:

CLOVE OIL

PROTOCOL:

Clove oil (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered, with water as the diluent, by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3).

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment. In addition, a small number of pierced and drilled controls were collected in some experimental groups.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated. This number has not been corrected for the sterile eggs or the eggs discarded due to accidents, thus providing a slightly higher mortality rate and a lower abnormality rate than was the actual case.

Column 4 is the percent mortality, i.e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

The comparable data for the solvent treated eggs and the pierced and drilled controls as well as the untreated controls are included in columns 3 through 6.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

Clove oil was found to be quite embryotoxic when administered to the embryos under all conditions of the test. In the air-cell treated embryos, toxicity was significantly (P=0.05) greater than solvent-treated eggs at all dose levels tested except 0.25 mg/egg at 0 hours. Yolk treatment, however, was better tolerated; at 0 hours, toxicity was significant at all dose levels above and including 1.0 mg/egg. At 96 hours the similar threshold dose was 0.5 mg/egg.

Probit analysis resulted in an LC_{50} of 0.132 mg/egg and 0.064 mg/egg with the air-cell treated embryos at 0 and 96 hours, respectively (Tables 1 and 2). The LC_{50} of yolk treated at 0 and 96 hours were 0.028 mg/egg and 0.017 mg/egg, respectively (Tables 3 and 4).

Abnormal birds were seen under all four conditions of the test, but, except in one instance, the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from the solvent background (P=0.05). (Of the 50 untreated control birds, only one was abnormal with curled toes which was a frequently seen minor abnormality.)

AIR CELL AT 0 HOURS: Abnormalities were found in all the groups treated with 0.5 mg/egg or less although the only level that was significantly different in frequency of abnormalities from the solvent-treated was 0.25 mg/egg where four animals showed curled toes. At 0.5 mg/egg, seven birds showed one or more of the following: hip contracture, talipes, abnormal feather growth. At 0.1 mg/egg there was one bird with celosomia and another one with hip contracture. The solvent-treated birds also had four abnormal birds with one or more of the following: celosomia, curled toes, genu valgus.

AIR CELL AT 96 HOURS: At 5.0 mg/egg, one bird showed no feather development on the head. This condition plus curled toes were seen in one bird at 0.1 mg/egg. One bird from the solvent-treated also had curled toes.

YOLK AT 0 HOURS: Two dosage levels, 1.0 mg/egg and 0.25 mg/egg, had one bird each showing contracture of the hips. No abnormalities were found with the solvent-treated birds.

YOLK AT 96 HOURS: There was a total of four abnormal birds, all with treated groups. One bird at 1.0 mg/egg had curled toes, and one bird with buphthalmia was found at 0.5 mg/egg. Two birds, one with celosomia and one with contracture of the ankle, were observed at 0.25 mg/egg. At no level was the frequency of the abnormality significantly different from the solvent treated group.

From the above observations, the teratogenicity of clove oil cannot be ascertained clearly. At only one dose level (0.25 mg/egg air cell at 0 hours) was the percentage of abnormality significantly different from that of the solvent control. Most of the abnormalities described above were on the lower extremity and they were of the same varieties that were seen with the control animals.

Microscopical examination of the paraffin embedded and H&E stained sections revealed no consistent histological changes in any of the organs observed. Although occasional hemorrhage, vacuolization, or fatty infiltration in the liver were seen, neither of these changes correlated with the administered dose nor the varieties of the external abnormalities.

1. Oil of Clove, U.S.P. extra, S/S 7137, Lot #AB 4301, Fritzsche Dodge & Olcott, Inc., New York, N.Y., FDA 71-30

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963

3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964

4. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Clove Oil

Air Cell at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20.0	400	75	100*	0	0
10.0	200	84	100*	0	0
5.0	100	80	100*	0	0
1.0	20	100	98.00*	0	0
0.50	10	93	81.72*	8.60	7.52
0.25	5	14	57.14	35.71*	28.57*
0.10	2	21	71.42*	14.28	9.52
Water		108	34.25	4.62	3.70
Drilled control		35	34.28	0	0
Control		50	30.00	2.0	2.0

LC_{30} 0.060 mg/egg (1.200 mg/kg)

LC_{50} 0.132 mg/egg (2.648 mg/kg)

LC_{90} 0.914 mg/egg (18.298 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 2

Clove Oil

Air Cell at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20.0	400	81	100*	0	0
10.0	200	74	100*	0	0
5.0	100	73	98.63*	1.36	1.36
1.0	20	81	100*	0	0
0.50	10	86	100*	0	0
0.25	5	15	86.66*	6.66	0
0.10	2	26	65.38*	11.53	3.84
Water		86	36.04	1.16	1.16
Drilled control		27	25.92	0	0
Control		50	30.00	2.00	2.00

LC_{30} 0.037 mg/egg (0.675 mg/kg)

LC_{50} 0.064 mg/egg (1.281 mg/kg)

LC_{90} 0.306 mg/egg (6.130 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 3

Clove Oil

Yolk at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20.0	400	34	100*	0	0
10.0	200	37	100*	0	0
5.0	100	32	96.87*	0	0
1.0	20	52	96.15*	1.92	1.92
0.5	10	53	86.79	0	0
0.25	5	18	83.33	5.55	5.55
0.10	2	17	82.35	0	0
Water		20	65.00	0	0
Pierced control		28	71.42	0	0
Control		50	30.00	2.00	2.00

LC_{30} 0.005 mg/egg (0.114 mg/kg)

LC_{50} 0.028 mg/egg (0.570 mg/kg)

LC_{90} 1.457 mg/egg (29.156 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 4

Clove Oil

Yolk at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20	400	30	100*	0	0
10	200	38	100*	0	0
5.0	100	33	96.96*	0	0
1.0	20	56	96.42*	1.78	1.78
0.5	10	54	92.59*	3.70	1.85
0.25	5	24	79.16	12.50	8.33
0.10	2	22	68.18	0	0
Water		30	73.33	0	0
Pierced Control		13	23.07	0	0
Control		50	30.00	2.00	2.00

LC_{30} 0.003 mg/egg (0.066 mg/kg)

LC_{50} 0.017 mg/egg (0.350 mg/kg)

LC_{90} 1.023 mg/egg (20.473 mg/kg)

*Significantly different from solvent $P \leq 0.05$